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1: Biochemistry. 1997 Oct 7;36(40):12147-54.

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### Random mutagenesis of the poly(ADP-ribose) polymerase catalytic domain reveals amino acids involved in polymer branching.

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Poly(ADP-ribose) polymerase (PARP) is a multifunctional nuclear zinc finger protein which participates in the immediate response of mammalian cells exposed to DNA damaging agents. Given the complexity of the poly(ADP-ribosylation) reaction, we developed a large-scale screening procedure in *Escherichia coli* to identify randomly amino acids involved in the various aspects of this mechanism. Random mutations were generated by the polymerase chain reaction in a cDNA sequence covering most of the catalytic domain. Out of 26 individual mutations that diversely inactivated the full-length PARP, 22 were found at conserved positions in the primary structure, and 24 were located in the core domain formed by two beta-sheets containing the active site. Most of the PARP mutants were altered in poly(ADP-ribose) elongation and/or branching. The spatial proximity of some residues involved in chain elongation (E988) and branching (Y986) suggests a proximity or a superposition of these two catalytic sites. Other residues affected in branching were located at the surface of the molecule (R847, E923, G972), indicating that protein-protein contacts are necessary for optimal polymer branching. This screening procedure provides a simple and efficient method to explore further the structure-function relationship of the enzyme.

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